EFFICACY REVIEW

Product: **Bromadiolone Bait Bar, Bromadiolone 2.5% Concentrate, and Bromadiolone Technical**

Date: September 16, 2003

EPA File Symbol(s): 63823-LE, 63823-LG, and 63823-LN

DP Bar code(s): D287415, D287420, and D287408

Chemical Code: 112001 Bromadiolone

Formulation(s): Bromadiolone Bait Bar, a 99% Bromadiolone Technical, and a 2.5% Bromadiolone Concentrate.

Purpose for Review: The purpose for this review is to determine if the Bromadiolone Bait Bar and the asscoiated 2.5% Bromadiolone Concentrate are efficacious as a rat or mouse control product.

MRID(s):

45810809 J. Mach. 2002. Norway Rat (*Rattus norvegicus*) Anticoagulant Wax Block Laboratory Test Using Bromadiolone Bait Bar, Containing 50 PPM Bromadiolone, Formula # 210: Three-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01015. 125pp.

45810810 J. Mach. 2002. Norway Rat (*Rattus norvegicus*) Acute Dry Bait Laboratory Test Using Bromadiolone Bait Bar, Containing 50 PPM Bromadiolone, Formula # 210: One-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01016. 128pp.

45810811 J. Mach. 2002. House Mouse (*Mus musculus*) Anticoagulant Wax Block Laboratory Test Using Bromadiolone Bait Bar, Containing 50 PPM Bromadiolone, Formula # 210: Three-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01017. 115pp.

45810812 J. Mach. 2002. House Mouse (*Mus musculus*) Acute Dry Bait Laboratory Test Using Bromadiolone Bait Bar, Containing 50 PPM Bromadiolone, Formula # 210: One-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01018. 122pp.

45810813 J. Mach. 2002. Norway Rat (*Rattus norvegicus*) Anticoagulant Wax Block Laboratory Test Using "Weathered" LX-125 Bromadiolone Bait Bar, Containing 50 PPM Bromadiolone, Formula # 210: Three-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01042. 124pp.

45810814 Anticoagulant Wax Block Laboratory Test Using "Weathered" LX-125 Bromadiolone Bait Bar, Containing 50 PPM Bromadiolone, Formula # 210: Three-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01043. 100pp.

Good Laboratory Practices: Yes

Branch Chief: Meredith Laws

Team Leader: John Hebert, Product Manager - 07

IRB Reviewer: Geraldine R. McCann, Environmental Protection Specialist

BACKGROUND: **Landis International** has applied for a new product registration for Bromadiolone Bait Bar, a Bromadiolone Technical, and a Concentrate to control rats and mice. The efficacy tests associated with these products were conducted according to the Product Performance guidelines 96-10: Commensal Rodenticides: OPP Designation 1.209: Norway Rat/Roof Rat Acute Dry Bait Laboratory Test Method; OPP Designation 1.210: Standard House Mouse Acute Dry Bait Laboratory Test Method; OPP Designation: 1.213: Standard Norway Rat Anitcoagulant Wax Block and Wax Pellet Laboratory Test Method; and OPP Designation: 1.214: Standard House Mouse Anticoagulant Wax Block and Wax Pellet Laboratory Test Method, respectfully. This is a review of six efficacy tests and the Bromadiolone Bait Bar label.

REVIEW OF DATA:

1. **45810809** J. Mach. 2002. Norway Rat (*Rattus norvegicus*) Anticoagulant Wax Block Laboratory Test Using Bromadiolone Bait Bar, Containing 50 PPM Bromadiolone, Formula # 210: Three-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01015. 125pp.

DISCUSSION: This study was conducted to determine the efficacy of a Bromadiolone Bait Bar formulated to contain 50 ppm bromadiolone for control of male and female Wistar albino rats in a three-day feeding test.

The bromadiolone bait used in this test was formulated from the 2.5% Bromadiolone Concentrate. The formulation was mixed and extruded at Genesis Laboratories, Inc. into a two inch bar that was cooled and cut into 2 inch length pieces with a miter saw. In Appendix D5, Test Substance Raw Data (page 118), the Bromadiolone Bait Bars were formulated at Genesis Laboratories on August 25, 2001. To ensure that formula # 210, used in the test, is the formula for the proposed Bromadiolone Bait Bar product, a list of ingredients is needed to compare the products. The Genesis Laboratories, Inc. log number was recorded as a reference number to the bait ("01-TS-33"). The OPP rat and mouse challenge diet formulation is referenced as an SOP LR 4.02.

Rats arrived at the test facility September 10, 2001. They were acclimated to the facility (7 days), to the test room (10 days), and testing began September 21, 2001. The test was structured to fit a "three-day schedule", NOT part of the OPP guideline 1.213, which states "Maintain test period for 15 days." (OPP 1.213, 7.1).

The animals were weighed a week after they arrived (09/17/2001) and again 3 days before (09/21/2001) the testing began (09/24/2001). The allowable difference between the average pretest weights for the male and female rats should be within 50 grams. The average difference in this group of male and female rat weights pretest was 45.8 grams.

Bait acceptance for the two treated groups combined was 35.4%. The rats were presented with approximately 40 g of the Bromadiolone Bait Bar and 50 g of the OPP rat and mouse challenge diet each day (24 hours) for three days. The test ran for 10 days posttreatment. One male survived from Replicate I (16). No control animals died. The label specifies two (2) to eight (8) pieces of block at each placement for rats. This is 113.4 g to 453.6 g to be placed uninterrupted for 10 days. When testing a particular product, the application rate stated on the label should be the application tested.

Paper plates were placed beneath the feeding area of the rats to catch any spilled toxic bait or challenge diet. There was no discussion about weighing back the spillage, just that it was figured into the equation. Since this was a three-day test, there may be issues surrounding reissuing the dishes back to the animals. The use of "jars" to describe the feeders is also questionable since the guidelines call for metal or ceramic feeders designed so that rats may not nestle or wallow in the diet. A stainless steel automatic watering system was used instead of the gravity fed or open cup waterers as specified in OPP guideline 1.213, 6.2.

The guidelines for the test specify the temperature should range from 20 to 25 °C and the humidity should be between 50 and 55 % (OPP guideline 1.213, 5.1). The temperature for this test ranged from 18 to 25 °C pre-test and 19 to 25 °C during the test. The humidity ranged from 23 to 70 % pre-test and 29 to 65% during the test period. More consistency is needed in maintaining the temperature and humidity when conducting these laboratory tests.

The first animal to die was 102, Replicate II, female. She died on day 3. The deviations in the study found on page 34 of 125, mention that rats 51 and 56 failed to receive paper plates for spillage on 9/29/2001. Animal 51 was in the female control group and 61 is in the Replicate 1 males group. No food weights were recorded and cannot be calculated for rat 16 (male survivor) on days 5 and 6 of the test and rat 141 (female control animal) on days 8 and 9.

Mortality of the test animals was 97.5 %. Results of the rat test are summarized below:

Table 1. Rep I - Rats on Bromadiolone Bait Bar
Pretest Weights Bait 3-Day Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Treated Bait Consumed (g)	Total Bait Consumption (g)
M (10)	264.51	1,251.7	710.80	1,962.5
F (10)	221.13	**95% Mortality**		Percent **Bromadiolone Bait** Consumed **36.4 %**
Total (20)	Group Difference 43.38			

Table 2. Rep II - Rats on Bromadiolone Bait Bar
Pretest Weights Bait 3-Day Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Treated Bait Consumed (g)	Total Bait Consumption (g)
M (10)	264.4	1218.9	621.9	1840.8
F (10)	216.2	**100% Mortality**		Percent **Bromadiolone Bait** Consumed **34.4 %**
Total (20)	Group Difference 48.2			

Table 3. Control Group Rats on OPP Challenge Diet 3-Day
Pretest Weights Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)
M (10)	262.1	1570.5
F (10)	216.1	**0% Mortality**
Total (20)	Group Difference 46.0	

2. **45810810** J. Mach. 2002. Norway Rat (*Rattus norvegicus*) Acute Dry Bait Laboratory Test Using Bromadiolone Bait Bar, Containing 50 PPM Bromadiolone, Formula # 210: One-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01016. 128pp.

DISCUSSION: This study was conducted to determine the efficacy of Bromadiolone Bait Bars formulated to be a 50 ppm bromadiolone product for control of male and female Wistar albino rats in a one-day feeding test.

On Page 3 of 128, the Good Laboratory Practice Statement page, a reference was made to the bait weight: "The test substance was received on 8/25/01 and was assigned 01-TS-33. Two samples were removed before this period for analysis and a Sponsor sample (2 blocks for each sample @ ~40 grams per block = ~160 grams). The test substance was not weighed until 9/5/01. An account of the exact amount of bait remove is not available, however, based upon the mean weight of each block, we can be estimate that ~160 grams of bait was removed."

The bromadiolone bait used in this test was formulated from the 2.5% Bromadiolone Concentrate. The formulation was mixed and extruded at Genesis Laboratories, Inc. into a two inch bar that was cooled and cut into 2 inch length pieces with a miter saw. In Appendix D5, Test Substance Raw Data (page 118), the Bromadiolone Bait Bars were formulated at Genesis Laboratories on August 25, 2001. To ensure that formula # 210, used in the test, is the formula for the proposed Bromadiolone Bait Bar product, a list of ingredients is needed to compare the products. The Genesis Laboratories, Inc. log number was recorded as a reference number to the bait ("01-TS-33"). The OPP rat and mouse challenge diet formulation is referenced as an SOP LR 4.02.

Rats arrived at the test facility September 10, 2001. The testing began September 24, 2001. The study followed Subdivision G (Product Performance) Guideline 96-10: Commensal Rodenticides, and the OPP guideline 1.209 (Standard Norway Rat/Roof Rat Acute Dry Bait Laboratory Test Method).

This test was run for 1 day, as recommended in the OPP guideline 1.209, 1., for "single-feeding" claims for second generation anticoagulants. The 10-day post treatment observation was extended to 13 days "The rats were maintained 13 days following the 1-Day test or until death."

The rats were presented with 40 g of the bait bar and 50 g of the OPP rat and mouse challenge diet for 24 hours. The label states: "Apply two to eight 2-oz pieces (113.4 g to 453.6 g) per placement for rats. For mice, break block and apply one 2-oz piece (56.7 g) per placement." Paper plates were placed beneath the feeding area of the rats to catch any spilled bait or challenge diet. There was no discussion about weighing back the spillage, just that it was figured into the equation. Toxic bait acceptance for the two treated groups combined was 20.05%. The use of glass "jar" feeders is also questionable since the guidelines call for metal or ceramic feeders designed to the rats may not nestle or wallow in the diet. A stainless steel automatic watering systems was used instead of the gravity fed or open cup waterers as specified in the (OPP guidelines 1.209,6.2).

The guidelines for the test facility specify the temperature should range from 20 to 25 °C and the humidity should be between 50 and 55 % (OPP guideline 1.209, 5.1). The temperature for this test ranged from 18 to 25 °C pre-test and 19 to 25 °C during the test. The humidity ranged from 23 to 70 % pre-test and 29 to 66% during the test period. More consistency should be sought when conducting these

laboratory tests.

The difference between the average pretest weights for the male and female rats should be within 50 g and the average difference between the male and female weights pretest for this group was 47.4 g. The males in the test weighed an average of_ = 262.46 g and the females averaged _ = 215.05 g.

Mortality in both treated groups was 90 % for anticoagulant bait. The control group did not experience mortality. Results of the rat test are summarized below:

Table 1. Rep I - Rats on Bromadiolone Bait Bar
Pretest Weights 1-Day Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Bait Bar Consumed (g)	Total Bait Consumption (g)
M (10)	259.84	418.7	121.0	539.7
F (10)	215.90	**100% Mortality**		Percent **Toxic Bait** Consumed **22.95%**
Total (20)	Group Difference 43.94			

Table 2. Rep II - Rats on Bromadiolone Bait Bar
Pretest Weights 1-Day Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Bait Bar Consumed (g)	Total Bait Consumption (g)
M (10)	264.58	446.1	91.5	537.6
F (10)	213.20	**95% Mortality**		Percent **Toxic Bait** Consumed **17.15%**
Total (20)	Group Difference 51.38			

Table 3. Contol Rats on OPP Challenge Diet
Pretest Weights Bait Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)
M (10)	262.06	1579.5
F (10)	216.06	**0% Mortality**
Total (20)	Group Difference 46.0	

3. **45810811** J. Mach. 2002. House Mouse (*Mus musculus*) Anticoagulant Wax Block Laboratory Test Using Bromadiolone Bait Bar, Containing 50 PPM Bromadiolone, Formula # 210: Three-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01017. 115pp.

DISCUSSION: This study was conducted at Genesis Laboratories, Inc. in Wellington, Colorado, to determine the efficacy of a Bromadiolone Bait Bar formulated to be a 50 ppm bromadiolone product for control of male and female house mice in a three-day feeding test.

On Page 3 of 115, the Good Laboratory Practice Statement page, made a reference to the bait weight: "The test substance was received on 8/25/01 and was assigned 01-TS-33. The test substance was not weighed until 9/5/01. Two samples were removed before this period for analysis and a Sponsor sample (2 blocks for each sample @ ~40 grams per block = ~160 grams). An account of the exact amount of bait remove is not available, however, based upon the mean weight of each block, we can be estimate that ~160 grams of bait was removed." Appendix D5, Test Substance Raw Data (page 107), confirms that the bromadiolone bait was initially logged onto the form on September 5, 2001. Formulation and extrusion of the baits took place at Genesis Laboratories, Inc. The extruder/packer was made by "The Bonnet Company". The OPP rat and mouse challenge diet formulation is referenced as an SOP LR 4.02.

The introduction to this study refers to a "mortality rate of 90% or greater following a 3-day exposure period and a minimum of 33% of the test substance acceptance is the criteria...". This study did not follow the guidelines. OPP guideline 1.214 is a 15-day study involving weathered bait with 5 days posttreatment feeding. I recommend that OPP Guideline 1.204 be followed for the next test of this nature; however, and it is also a 15-day test.

One hundred and fifty house mice arrived at Genesis Laboratories, Inc. on September 10, 2001. The mice were assigned cages (972 cm^2) in same sex groups of 5 and were held in pretest holding and acclimation for 14 days (9/10/2001 to 9/24/2001). The housing for the caged mice was less than half the recommended size according to the OPP guidelines 1.214, 3.1. The recommended size is 2000 cm^2. Only 1 shelter was provided instead of the 3 shelters called for in the guidelines. The test dishes, were metal and the challenge dishes contained a "food follower."

The mice groups were randomly selected out of the larger population and weighed pretest, then held for 3 more days of acclimation. Testing began September 24, 2001, and ended October 7, 2001. The difference between the average weight of the female house mice (22.7 g) compared to the average weight of the male house mice (26.3 g) pretest was 3.57 g, an acceptable margin (5 g or less). The female weights ranged from 19.5 g to 29.2 g and the males ranged from 22.1 to 30.2 g.

The test room temperature is to be within 20 to 25 °C (OPP guideline 1.214) and the actual temperature ranged from 19 to 25 °C during the pretest holding and testing. The range in temperature is acceptable. The guidelines also specify the humidity in the test room to range between 50 and 55 % relative humidity. The humidity recorded in the test room at Genesis Laboratories, Inc. during the acclimation period was 29 to 70 %. The humidity during the test was 27 to 71% and no raw data was presented to show the daily recording data. Some form of regulated humidification must be made available in these test rooms. The temperature and humidity were not recorded on 9/22/2001. It was an inadvertent error pre-test. The timed lighting of 12 hours dark and 12 hours light had a light intensity of 8.7 foot candles measured by a NIST traceable Extech Instruments model LM-1 light meter.

Water must be available to each animal at all times. Glass water bottles equipped with ball-type watering tubes are recommended. Gravity fed automatic or open-cup type waterers are not recommended (OPP guideline 1.214, 6.2). Glass water bottles with sipper tubes were used for this test. It is not known if the sipper tubes had a ball-type device.

The mouse groups were fed "approximately 40 g of test substance (the label states to feed 1-2 oz block, approximately 113.4 g) and approximately 50 g of challenge diet in the other cup" (page 11 of 115). OPP guidelines 1.214, 6.3 states that "Containers must be identical in size and type and must be placed equidistant from the sides of the cage and equidistant from the rodent's point of access to water." The rat and mouse challenge dishes in this test had "Food followers" placed on top to help control spillage. This may explain why the mice ate more of the bait bar than the rat and mouse challenge bait. They had no barrier on the bait bar and limited access to the challenge diet.

On page 11 of 115, the last paragraph " Spilled diet was collected by placing a 9 in diameter paper plate under each cup of each cage. At each 24-hour interval, the cups were removed, spillage was retrieved, and consumption was weighed to the nearest 0.1 gram." Was the spillage weighed and discarded or placed back in the test dishes and weighed? It is not known what happens from reading the study report.

OPP 1.214, 7.1 states "Maintain test period for 15 day. Monitoring of all surviving animals (including those in the control group) must continue for the 15-day baiting period plus the 5-day post baiting follow-up period, even if 100% mortality of test mice is recorded before the 20 days have elapsed." The test lasted from 9/24/2001 to 10/7/2001 (13 days), 3 test days and 10 posttreatment.

No claims on the label refer to the bait as "resistant to mold" or effective in "wet or damp areas" so running the test replication without a weathered bait is acceptable (1.214, 7.5).

Bait acceptance for the two treated groups combined was 53.25 %. For this test to be acceptable, at least 90 % of the test subjects must die during the bait exposure and post exposure observation periods. Cage ll females had 1 survivor. Overall mortality for the three test groups was 97.5 %.

Results of the mouse test is summarized below:

Table 1. REP I - Mice on OPP Challenge Diet and Bromadiolone Bait Bar - 3-Day Pretest Weights Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Toxic Bait Consumed (g)	Total Bait Consumption (g)
M (10)	26.01	122.9	128.0	250.9
F (10)	23.98	**95% Mortality**		Percent Toxic Bait Consumed **51%**
Total (20)	Group Difference 2.03			

Table 2. REP II - Mice on OPP Challenge Diet and Bromadiolone Bait Bar- 3-Day Pretest Weights Test Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Toxic Bait Consumed (g)	Total Bait Consumption (g)
M (10)	26.92	117.8	153.0	270.8
F (10)	22.25	**100 % Mortality**		Percent Pelleted Consumed **56.5%**
Total (20)	Group Difference 4.67			

Table 3. Mice on Control Bait
Pretest Weights Bait Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)

M (10)	25.95	177.2
F (10)	21.94	**0 % Mortality**
Total (20)	Group Difference 4.01	

4. **4.45810812** J. Mach. 2001. House Mouse (*Mus musculus*) Acute Dry Bait Laboratory Test Using Bromadiolone Bait Bar, Containing 50 ppm Bromadiolone, Formula #210: One-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01018. 122pp.

DISCUSSION: This study was conducted at Genesis Laboratories, Inc. in Wellington, Colorado, to determine the product efficacy of a Bromadiolone Bait Bar formulated to a 50 ppm bromadiolone for control of male and female house mice in a one-day feeding test.

On Page 3 of 122, the Good Laboratory Practice Statement page, made a reference to the bait weight: "The test substance was received on 8/25/01 and was assigned 01-TS-33. The test substance was not weighed until 9/5/01. Two samples were removed before this period for analysis and a Sponsor sample (2 blocks for each sample @ ~40 grams per block = ~160 grams). An account of the exact amount of bait remove is not available, however, based upon the mean weight of each block, we can be estimate that ~160 grams of bait was removed." Appendix D5, Test Substance Raw Data (page 114), confirms that the bromadiolone bait was initially logged onto the form on September 5, 2001. Formulation and extrusion of the baits took place at Genesis Laboratories, Inc. The extruder/packer was made by "The Bonnet Company"

The introduction states that the OPP guideline 1.210: Standard House Mouse Acute Dry Bait Laboratory Test Method is being followed. This is the "one-day" version of this test used to establish the "single-feeding" claim for a second generation anticoagulant. The author states "Although the active ingredient, bromadiolone, is not an "acute" toxicant per se, the same guideline applies for second generation and later anticoagulants in order to establish a single-feeding efficacy claim." The OPP rat and mouse challenge diet formulation is referenced as an SOP LR 4.02.

One hundred and fifty house mice (75 males and 75 females) arrived at Genesis Laboratories, Inc. on September 10, 2001. The mice were randomly assigned to ¼" to ½" wire mesh cages (972 cm^2) in same sex groups of 5 and were held in pretest holding acclimation for 14 days. The housing for the caged mice was less than half the recommended size according to the OPP guidelines 1.210, 3.1. The recommended size is 2000 cm^2.

The mice were weighed pretest (September 21, 2001) and then held for 3 more days of acclimation. The raw data reflect that the weights were taken on September 24th, 2001, and transcribed on September 24, 2001. This was properly documented. The difference between the average weight of the female house mice (23.02 g) compared to the average weight of the male house mice (26.28 g) pretest was 3.27 g, an acceptable margin (5 g or less).

In this test, the animals are to be provided with shelters. Documentation for this test states only one shelter was issued to groups of 5 mice. The OPP guideline 1.210, 3.3, states: "Use at least three shelters for subgroupings of five mice." The test room temperature is to be within 20 to 25 °C (OPP guideline 1.210) and were recorded daily with a digital thermometer/hygrometer from Fisher, Model 11-661-13. The actual temperature ranged from 19 to 24 °C during the pretest holding and testing. The range during the testing was 19 to 32 °C. The guidelines also specify the humidity in the test room to range between 50 and 55 % relative humidity. The humidity recorded in the test room at Genesis Laboratories, Inc. during the acclimation period was 29 to 70 %. The humidity during the test was 32 to 71%. No raw data was presented to show the daily recording data. Raw data must be presented in the future. A more stable form of regulated humidification must be made available for use in these test rooms.

The mouse groups were fed "approximately 40 grams of test substance and approximately 50 grams of challenge diet..." one-day choice test." (page 11 of 122). OPP guidelines 1.210, 6.3, states that "The food offered in each container should be equal and consistent throughout the test and must be a least 10 grams per container per animal per day." Mouse groups of 5 should have received a minimum of 50 grams of food in each cup. The label for the product specifies "two (2) pieces of block" or 113.4 g of bait for mice.

Water must be available to each animal at all times. Glass water bottles equipped with ball-type watering tubes are recommended. Gravity fed automatic or open-cup type waterers are not recommended (OPP guideline 1.210, 6.2). Glass water bottles with sipper tubes were used for this test. It is not known if the sipper tubes had a ball-type device.

There is no mention of drying the spillage before weighing it back or how the spillage was accounted for. Page 11 of 122 states that "Spilled diet was collected by placing a 9 inch diameter paper plate under each cup of each cage. Plate positions were reversed according to respective cup positions 12 hours after initial presentation. After 24 hours, the cups were removed, spillage was retrieved, and consumption was weighed to the nearest 0.1 gram." More clarification is needed about this practice of collecting spillage at this laboratory to determine if it affects the consumption. Was the spillage added back to the cups? Was it weighed separately, the weight added to the calculations, and discarded? How was the spillage handled?

Bait acceptance for the two treated groups combined was 31.0 %. For this test to be acceptable, at least 90 % of the test subjects must die during the bait exposure and post exposure observation periods. Mortality was 97.5 %.

Results of the mouse test are summarized below:

Table 1. REP I - Mice on OPP Challenge Diet and Bromadiolone

Pretest Weights **Bait Bar - 1-Day Test-Consumption and Mortality**

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Toxic Bait Consumed (g)	Total Bait Consumption (g)
M (10)	25.76	53.7	22.3	76.0
F (10)	23.68	**100% Mortality**		Percent Toxic Bait Consumed **29.3%**
Total (20)	Group Difference 2.08			

Table 2. REP II - Mice on OPP Challenge Diet and Bromadiolone Bait Bar - 1-Day Test-Consumption and Mortality

Pretest Weights

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Toxic Bait Consumed (g)	Total Bait Consumption (g)
M (10)	27.14	50.0	24.4	74.4
F (10)	23.43	**95% Mortality**		Percent Toxic Bait Consumed **32.8 %**
Total (20)	Group Difference 3.71			

Table 3. Mice on Control Bait

Pretest Weights **Bait Consumption and Mortality**

Sex	Average Group Weight (g)	OPP Diet Consumed (g)
M (10)	25.95	84.0
F (10)	21.94	**0 % Mortality**
Total (20)	Group Difference 4.01	

5. **45810813** J. Mach. 2002. Norway Rat (*Rattus norvegicus*) Anticoagulant Wax Block Laboratory Test Using "Weathered" LX-125 Bromadiolone Bait Bar, Containing 50 PPM Bromadiolone, Formula # 210: Three-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01042. 124pp.

DISCUSSION: This study was conducted to determine the efficacy of weathered Bromadiolone Bait Bars formulated to be a 50 ppm bromadiolone product for control of male and female Wistar albino rats in a three-day feeding test.

On Page 3 of 124, the Good Laboratory Practice Statement page, analysis of the bait was done before the Study Director signed the protocol. Study 01042/43 is an exact repeat of 01019/20. Formulation and extrusion of the bait blocks took place at Genesis Laboratories, Inc. into a two inch bar that was cooled and cut into 2 inch length pieces with a miter saw. The extruder/packer was made by "The Bonnet Company". The bromadiolone bait used in this test was formulated from the 2.5% Bromadiolone Concentrate. After the weathering process (October 28, 2001 til November 12, 2001) in a "Larry's Incubator", the test substance was stored under the assigned number: 01-TS-40. The bait bars developed white clumps of mold on day 5 and "greenish mold" about day 8 (page 115 of 124). In Larry's Incubator, using both dry and wet bulb temperatures, the temperatures ranged from 100 to 101°F and 97 to 99°F, respectively. The humidity ranged from 89% to 96%. The OPP rat and mouse challenge diet (formulated October 23,2001) formulation is referenced as an SOP LR 4.02.

Rats arrived at the test facility October 29, 2001. The testing began November 13, 2001, following Subdivision G (Product Performance) Guideline 96-10: Commensal Rodenticides, and the OPP guideline I.213 (Standard Norway Rat Anticoagulant Wax Block and Wax Pellet.Laboratory Test Method).

This study did not follow the guidelines it quoted. The OPP guideline 1.213 is a 15-day study involving weathered bait with 5 days posttreatment feeding. The rats in this test were fed for 3 days with 13 days posttreatment feeding and observation. According to the author, "The purpose of this study was to investigate the effectiveness of "weathered" LX-125 Bromadiolone Bait Bar" and to obtain "A mortality rate of 80% or greater following a 3-day exposure period" ... with "a minimum of 25% test substance acceptance." In fact, these are the "acceptable" study criteria for a 15 day study and a 5 day posttest observation period on weathered bait products.

The rats were presented with "at least 50 g" of the bait bar and "approximately 50 g" of the OPP rat and mouse challenge diet each day. The label states: "Apply two to eight 2-oz pieces (113.4 g to 453.6 g) per placement for rats. "Weathered" toxic bait acceptance for the two treated groups combined was 29.6%. Paper plates were placed beneath the feeding area of the rats to catch any spilled bait or challenge diet. There was no discussion about weighing back the spillage, just that it was figured into the equation. The use of glass "jar" feeders is also questionable, however, there is no specific guidance for using metal or ceramic feeders designed so the rats may not nestle or wallow in the diet in this particular OPP guideline (1.213). A stainless steel automatic watering systems was used instead of the gravity fed or open cup waterers as specified in the (OPP guidelines 1.213, 6.2).

As a side note, the data from the control group (Females #1, page 82 of 124), day 2 presentation of bait for day 3 was less than the removed bait from day 2. Same with #46, page 85. Was it spilled?

The guidelines for the test facility specify the temperature should range from 20 to 25 °C and the humidity should be between 50 and 55 % (OPP guideline 1.213, 5.1). The temperature for this test ranged from 20 to 26 °C pre-test and 20 to 25 °C during the test. The humidity ranged from 21 to 75 % pre-test and 30 to 70% during the test period. More consistency should be sought when conducting these laboratory tests.

The difference between the average pretest weights for the male and female rats should be within 50 g and the average difference between the male and female weights pretest for this group was 34.83 g. The males in the test weighed an average of_ = 267.5 g and the females averaged _ = 232.68 g.

Mortality in both treated groups was 95 %. The control group did not experience mortality. Results of the rat test are summarized below:

Table 1. Rep I - Rats on Weathered Bromadiolone Bait Bar
Pretest Weights 1-Day Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Bait Bar Consumed (g)	Total Bait Consumption (g)
M (10)	275.54	1,192.4	512.2	1,704.6
F (10)	234.67	**95% Mortality**		Percent **Toxic Bait** Consumed **30.0%**
Total (20)	Group Difference 40.87			

Table 2. Rep II - Rats on Weathered Bromadiolone Bait Bar
Pretest Weights 1-Day Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Bait Bar Consumed (g)	Total Bait Consumption (g)
M (10)	266.56	1,211.2	500.7	1,711.9
F (10)	227.35	**95% Mortality**		Percent **Toxic Bait** Consumed **29.2%**
Total (20)	Group Difference 39.21			

Table 3. Control Rats on OPP Challenge Diet
Pretest Weights Bait Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)
M (10)	260.42	1552
F (10)	236.02	**0% Mortality**
Total (20)	Group Difference 24.4	

6. **45810814** Anticoagulant Wax Block Laboratory Test Using "Weathered" LX-125 Bromadiolone Bait Bar, Containing 50 PPM Bromadiolone, Formula # 210: Three-Day Test. Genesis Laboratories, Inc. Unpublished Report. Study #01043. 100pp.

DISCUSSION: This study was conducted to determine the efficacy of weathered Bromadiolone Bait Bars formulated to be a 50 ppm bromadiolone product for control of male and female Wistar house mice in a three-day feeding test.

This study did not follow the guidelines it quoted. OPP guideline 1.214 is a 15-day study involving weathered bait with 5 days posttreatment feeding. The mice

in this test were fed for 3 days with 13 days posttreatment feeding and observation. According to the author, "The purpose of this study was to investigate the effectiveness of "weathered" LX-125 Bromadiolone Bait Bar" and to obtain "A mortality rate of 80% or greater following a 3-day exposure period" ... with "a minimum of 25% test substance acceptance." In fact, these are the "acceptable" study criteria for a 15 day study and a 5 day posttest observation period on weathered bait products.

On Page 3 of 100, the Good Laboratory Practice Statement page, analysis of the bait was done before the Study Director signed the protocol. Study 01042/43 is an exact repeat of 01019/20. Formulation and extrusion of the bait blocks took place at Genesis Laboratories, Inc. into a two inch bar that was cooled and cut into 2 inch length pieces with a miter saw. The extruder/packer was made by "The Bonnet Company". The bromadiolone bait used in this test was formulated from the 2.5% Bromadiolone Concentrate. After the weathering process (October 28, 2001 til November 12, 2001) in a "Larry's Incubator", the test substance was stored under the assigned number: 01-TS-40. The bait bars developed white clumps of mold on day 5 and "greenish mold" about day 8 (page 90 of 100). In Larry's Incubator, using both dry and wet bulb temperatures, the temperatures ranged from 100 to 101°F and 97 to 99°F, respectively. The humidity ranged from 89% to 96%. The OPP rat and mouse challenge diet (formulated October 23, 2001) formulation is referenced as an SOP.

Seventy house mice (35 males and 35 females) arrived at Genesis Laboratories, Inc. on October 29, 2001. The mice were randomly assigned to ½" wire mesh cages (972 cm^2) in same sex groups of 5 and were held in pretest holding acclimation for 15 days. The housing for the caged mice was less than half the recommended size according to the OPP guidelines 1.214, 3.1. The recommended size is 2000 cm^2.

The mice were weighed on Day 0 of the test (November 13, 2001). The difference between the average weight of the female house mice (25.27 g) compared to the average weight of the male house mice (27.40 g) pretest was 2.14 g, an acceptable margin (5 g or less).

In this test, the animals are to be provided with shelters. Documentation for this test states only one shelter was issued to groups of 5 mice. The OPP guideline 1.214, 3.2, states: "Use at least three shelters for subgroupings of five mice."

The test room temperature is to be within 20 to 25 °C (OPP guideline 1.214) and were recorded daily with a digital thermometer/hygrometer from Fisher, Model 11-661-13. The actual temperature ranged from 20-24°C during the pretest holding and testing. The range during the testing was 21-25°C. The guidelines also specify the humidity in the test room to range between 50 and 55 % relative humidity. The humidity recorded in the test room at Genesis Laboratories, Inc. during the acclimation period was 22 to 70 %. The humidity during the test was 31 to 70%. No raw data was presented to show the daily recording data. Raw

data must be presented in the future. A more stable form of regulated humidification must be made available for use in these test rooms.

Water must be available to each animal at all times. Glass water bottles equipped with ball-type watering tubes are recommended. Gravity fed automatic or open-cup type waterers are not recommended (OPP guideline 1.214, 6.2). Glass water bottles with sipper tubes were used for this test. It is not known if the sipper tubes had a ball-type device.

The mouse groups were fed "...at least 100 grams of test substance and at least 100 grams of challenge diet in the respective cups." (page 11 of 100). There is no mention of drying the spillage before weighing it back or how the spillage was accounted for. Page 11 of 100 states that "Spilled diet was collected by placing a 9 inch diameter paper plate under each cup of each cage. At each 24-hour interval, the cups were removed, spillage was retrieved, and consumption was weighed to the nearest 0.1 gram." More clarification is needed about this practice of collecting spillage at this laboratory to determine if it affects the consumption. Was the spillage added back to the cups? Was it weighed separately, the weight added to the calculations, and discarded? How was the spillage handled?

Bait acceptance for the two treated groups combined was 38.0 %. For this test to be acceptable, at least 90 % of the test subjects must die during the bait exposure and post exposure observation periods. Mortality was 95.0 %.

Results of the mouse test are summarized below:

Table 1. REP I - Mice on OPP Challenge Diet and Weathered Bromadiolone
Pretest Weights Bait Bar - 1-Day Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Toxic Bait Consumed (g)	Total Bait Consumption (g)
M (10)	27.03	216.2	123.6	339.8
F (10)	24.06	**90% Mortality**		Percent Toxic Bait Consumed **36.4%**
Total (20)	Group Difference 2.97			

Table 2. REP II - Mice on OPP Challenge Diet and Weathered Bromadiolone
Pretest Weights Bait Bar - 1-Day Test-Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)	Toxic Bait Consumed (g)	Total Bait Consumption (g)
M (10)	26.77	157.1	105.2	262.3
F (10)	25.52	**100% Mortality**		Percent Toxic Bait Consumed **40.1 %**
Total (20)	Group Difference 1.25			

Table 3. Mice on Control Bait
Pretest Weights Bait Consumption and Mortality

Sex	Average Group Weight (g)	OPP Diet Consumed (g)
M (10)	28.41	84.0
F (10)	26.22	**0 % Mortality**
Total (20)	Group Difference 2.19	

Efficacy Comments

1. The introduction for the studies state that the OPP guidelines were being followed, but four of the tests (MRID 45810809, 45810811, 45810813, and 45810814) were 15-Day tests used as 3-day tests with 10 to 13 days posttreatment observation instead of 5 days. These are not guideline studies and are considered substandard.

2. The use of glass feeders or "jars" to issue the test substance or rat and mouse challenge diet is questionable since the guidelines call for metal or ceramic feeders designed so the rats or mice may not nestle or wallow in the diet.

3. A stainless steel automatic watering systems was used in several of the studies instead of gravity fed waterers as specified in the OPP guidelines cited above. Did the sipper tubes used have a ball mechanism?

4. The rats in study #01016 (MRID 45810810) were presented with 50 g of OPP rat and mouse challenge diet and 40 g of the bait bar. In study #01018 (MRID 45810812), the mice were fed with 50 g of OPP rat and mouse challenge diet and 40 g of the bait bar contrary to the OPP guidelines 1.209, and 1.210, respectfully, which read: "Containers must be identical in type and size..." and "The food offered in each container should be equal and consistent throughout the test and must be a least 15 grams per container per animal per day."

5. The OPP guidelines specify the temperature range from 20 to 25 °C and the humidity should be between 50 and 55 % in the test facility. In all of the tests, the temperature and humidity were not consistent and seemed out of control. More consistency should be sought when conducting these laboratory tests even though they were consistently out of control. A better form of regulated temperature and humidification must be made available for use in the test rooms.

6. No raw data was presented to show the daily temperature and humidity data. In the future, please provide the raw data from the daily readings of temperature and humidity from the test rooms for

each.

7. The housing for the caged mice was less than half the recommended size according to OPP guidelines 1.209, 3.1; 1.213, 3.1 for rats and 1.210, 3.1, and 1.214, 3.1 for mice. The recommended size for both is 2000 cm^2.

Efficacy Comments (cont.)

8. Please clarify the practice of collecting spillage by giving a written description of the process. Was the spillage added back to the cups? Was it weighed separately, discarded, and the weight added to the calculations?

9. Please provide a list of ingredients used for the toxice bait made for MRIDs 45810809 and 45810810. It is needed to verify the Bromadiolone Bait Bar product was used for these tests.

10. Provide a copy of the raw data for mixing the OPP rat and mouse challenge diet for the studies listed in this review.

Conclusion(s): The above mentioned studies were reviewed and found marginally acceptable if the requested additional information is acceptable. The deviations from the guidelines, omission of critical raw data, and not conforming to guideline specifications make these studies substandard. If these unacceptable practices mentioned above are continued in the future, studies will be rejected.

Label Comments

1. On the front of the label, replace "Kills" in the third line with "Controls" in it's place.

2. In the **SELECTION OF TREATMENT AREAS** section: the phrase: "...in or beside burrows,..." suggests that this bait may be used in a field situation. Also, the following phrase is too vague and ambiguous and should be deleted: "...in corners and concealed places,..." This statement should read: "Generally, these areas are along walls by gnawed openings, in or beside burrows within 15 feet of a building or wall, between floors and walls, or in locations where rodents or their signs have been seen."